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REVIEW ARTICLE

The Remarkable Biology of Pollen

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INTRODUCTION

To reproduce, higher plants utilize a unique multicellular microorganism: the male gametophyte, or pollen grain. The independent lifetime of flowering plant gametophytes is greatly abbreviated compared to that of gametophytes of more primitive plants, yet the angiosperm pollen grain must be able to survive at least briefly free from the sporophytic plant and perform a number of specialized functions before fertilization is accomplished.

Although the pollen grain is a rather simple two- or three-celled organism, cytogenetic and mutagenesis experiments indicate that higher plants make a significant investment in genetic material devoted to gametophyte production (Carlson, 1977; Birchler and Schwartz, 1979; Kindiger et al., 1991). Molecular studies identifying anther- and pollen-specific genes support the notion that the construction of a functional male gametophyte requires a rather large pool of such genes (Kamalay and Goldberg, 1980; Willing and Mascarenhas, 1984; McCormick, 1991). This is not so surprising given the specialized structures, mechanisms for rapid growth, and cell–cell communication systems during pollen–pistil interactions that have evolved to allow for both the survival of the gametophyte free of the sporophyte and the efficient delivery of sperm to the embryo sac.

Studies of certain aspects of the molecular mechanisms of gametophyte development and function have progressed very rapidly. Examples of these include the identification of *cis*-acting elements determining tissue-specific gene expression in pollen and tapetal cells (Ursin et al., 1989; Koltunow et al., 1990; McCormick et al., 1991) and of mechanisms of self-incompatibility in pollen–pistil interactions (McClure et al., 1989, 1990; Thorsness et al., 1991). The intent of this article is to focus on a few less well studied but intriguing aspects of pollen development and function, with an emphasis on pollen development in maize. For more general reviews of microsporogenesis, the reader is referred to Giles and Prakash (1987) and Mascarenhas (1989). For recent reviews of gene expression during pollen development, see Mascarenhas (1990) and McCormick (1991).

OVERVIEW OF POLLEN DEVELOPMENT

Pollen development takes place within the anther. Four anther wall layers (the epidermis, the endothecium, the middle layer, and the tapetum) enclose the fluid-filled locule, as shown in Figure 1. This locule contains the sporogenic cells that will undergo meiosis. The layer of anther cells adjacent to the locule is known as the tapetum, which is a tissue that is intimately involved in microsporogenesis (see below). Figure 2 summarizes pollen development in maize. At 50 to 60 days after seeding, the microsporocytes are encased in an impermeable β-1,3-glucan (callose) wall (Figure 2A), which effectively isolates the meiocytes from other cells (Knox and Heslop-Harrison, 1970). Each microsporocyte undergoes two meiotic divisions over a period of approximately 3 days, producing a tetrad of four haploid cells called microspores that are still encased within a callose wall (Figure 2B). After dissolution of the callose wall, the free young microspores grow rapidly for about 5 days. During this period, the outer pollen wall, or exine, is synthesized (Figure 2C). The centrally located nucleus migrates toward the cell periphery to a position opposite the pollen pore.

As the young microspores grow, they fill with multiple small vacuoles (Figure 2D) that eventually coalesce into a single large vacuole, compressing the cytoplasm into a small region opposite the pollen pore (Figure 2E). About 5 days after meiosis, the asymmetric division called microspore mitosis occurs, producing two cells with very different fates (Figure 2F). The bicellar product of microspore mitosis is, by definition, pollen. During the following 7 days, further maturation steps occur. In maize, the generative cell within the young pollen divides again to form two sperm cells (Figure 2G); in many other plant species, this second mitosis takes place only after germination of the pollen tube. The pollen grain then secretes the cellulosic and pectic inner pollen wall, or intine. The pollen grain accumulates starch granules until the grain is entirely engorged (Figure 2H). It appears that some mRNAs synthesized at this time may be stored for translation during germination (Mascarenhas et al., 1984; Mascarenhas, 1990). The pollen dehydrates, reducing the water content to 40 to 58% at the time of dehiscence (Barnabas, 1985). Further

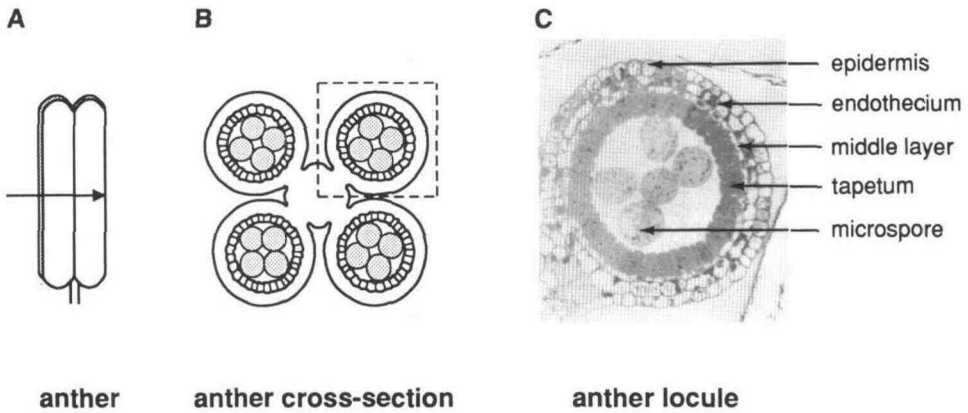

Figure 1. Structure of Maize Anther.

(A) Diagram of anther. The arrow indicates the plane of the cross-section shown in **(B)**.
(B) Diagram of cross-section of the anther. The dotted box indicates a single anther locule.
(C) A micrograph of an anther locule, with cell layers identified.

dehydration of the pollen grain after its release from the anther may cause domains of the vegetative cell plasma membrane to enter an unstable gel/liquid crystal state (Kerhoas et al., 1987). The maize pollen grain hydrates upon interaction with the silk, and a pollen tube is rapidly germinated through the pore to transport the sperm to the embryo sac.

THE TAPETUM: POLAR SECRETORY CELLS

As is the case for all higher organisms, the production of gametes in higher plants requires the participation of both the developing haploid gametophytic cells and accessory somatic (in the case of plants, sporophytic) diploid cells. The tapetum of higher plants plays a critical role in pollen development (Pacini et al., 1985). During meiosis, the tapetal cells undergo a dramatic differentiation into binucleate, polar secretory cells lacking a primary cell wall. Figure 3 shows that these cells are packed with ribosomes, mitochondria, endoplasmic reticulum, Golgi, and many vesicles on the locular face of the plasma membrane and are connected by cytoplasmic bridges early in development. The tapetum may remain associated with the anther wall (in which case it is termed secretory or parietal, as in maize), or the tapetal cells may actually fuse to form a syncytium that invades the anther locule, engulfing the developing microspores (in which case it is termed amoeboid, as in Tradescantia). Secretory vesicles are transported in a polar fashion to the locular surface of the cells.

Several tapetal cell functions are known. It has long been proposed that the tapetal cells play a nutritive role for the microspores, in direct analogy to nurse cells in mammalian systems. A second tapetal cell function is to release the young haploid microspores from the callose wall enclosing the meiotic tetrad by the secretion of a β-1,3-glucanase, or callase (Steiglitz, 1977). The timing of callase secretion appears to be critical for normal pollen development. In one case of cytoplasmic male sterility in petunia, the timing of callose wall dissolution is premature, leading to collapse of the developing microspores (Izhar and Frankel, 1971). This result has recently been repeated with a more defined system using transgenic plants. Worrall et al. (1992) fused a modified callase gene to an Arabidopsis promoter known to be active in the tapetum during meiosis (Scott et al., 1991; Paul et al., 1992). Partial or complete male sterility was observed in transgenic tobacco plants expressing the callase gene during meiosis. Meiosis was apparently normal in the absence of a callose wall, but pollen wall development was abnormal. Instead of a highly organized pattern of exine deposition, an electron-dense material that may be sporopollenin appeared to be randomly distributed on the microspore surface. How the presence of callose may contribute to the formation of a normal pollen wall remains to be clarified. A rather surprising result of this study was that the tapetal cells as well as the microspores exhibited abnormalities. This raises the intriguing possibility that the tapetal cells do not function independently of the microspores—in other words, the two cell types are somehow interdependent during pollen development.

A third proposed role for the tapetal cells is the production of precursors for the biosynthesis of the outer pollen wall, or exine. This aspect of tapetal cell function is described in more detail below. As the microspores become vacuolate, the tapetal cells undergo cell death. Even at this stage, the tapetal cells contribute to pollen development with the deposition of cell remnants called tryphine or pollenkitt on the maturing pollen surface. These substances are largely lipoidal in nature and may function to protect the pollen grain from dehydration or to allow it to attract and adhere to insect pollinators.

Other functions of the tapetum remain more mysterious. Genetic studies have identified recessive, sporophytic mutations at many different loci that cause male sterility (Beadle, 1932; Albertsen and Phillips, 1981; Kaul, 1988). Most cytological evidence points to the tapetal cells as the affected sporophytic cell type. This is almost certainly the case with cytoplasmic male sterility type T in maize, where irregularities in tapetal cell morphology are observed prior to pollen abortion (Warmke and Lee, 1977). It is possible that the majority of these mutations adversely affect pollen nutrition due to the malfunctioning of the tapetum. However, some male sterile (*ms*) mutations appear to have more specific phenotypic effects. Several of the male sterile mutants of maize (*ms7*, *ms1*) are defective in pollen wall biosynthesis, a finding consistent with the proposed role of the tapetum in exine production. Other *ms* mutations affect the progression of microspores through microspore mitosis, causing failure of chromosome condensation (*ms14*) or precocious chromosome condensation (*ms13*) (Albertsen and Phillips, 1981). The finding that a deficiency in a purine salvage pathway enzyme leads to abnormal pollen mitosis and male sterility in Arabidopsis (Regan and Moffatt, 1990) suggests that one way that chromosome behavior in microspores can be influenced is in the control of DNA precursor production.

A greater understanding of specific tapetal functions will be accomplished by isolating sporophytic male sterility genes. The generation of new male sterile mutants using transposable element–containing maize lines should allow the isolation of such genes through transposon tagging methods (P. Bedinger, A. Broadwater, C. Loukides and S. Stephenson, unpublished data). Similarly, T-DNA mutagenesis in Arabidopsis has identified several new male sterility genes that should be amenable to cloning (C. Makaroff and K. Feldmann, personal communication). Tomato YAC libraries, in conjunction with an extensive RFLP map, are currently being used to isolate male sterility genes by chromosome walking (S. McCormick, personal communication).

Tapetal cells provide an excellent target for the control of fertility through genetic engineering. Male sterility has been induced in tobacco through the selective destruction of the tapetum by fusing the promoter of a gene expressed specifically in tapetal cells to a cytotoxic ribonuclease gene (Mariani et al., 1990). Recent progress using a specific inhibitor of this ribonuclease to control its activity demonstrates the tremendous potential for the impact of biotechnology on agriculture, in this case, by providing a genetic means of producing hybrid seed (Mariani et al., 1992).

The importance of the tapetum in microsporogenesis, first suggested by cytological studies and now confirmed by molecular studies, is beyond dispute. What remains to be elucidated are the molecular mechanisms of tapetal function. How is the dramatic differentiation of the tapetum during meiosis controlled? What are the different specific roles of the tapetum during microsporogenesis? Is there a two-way communication

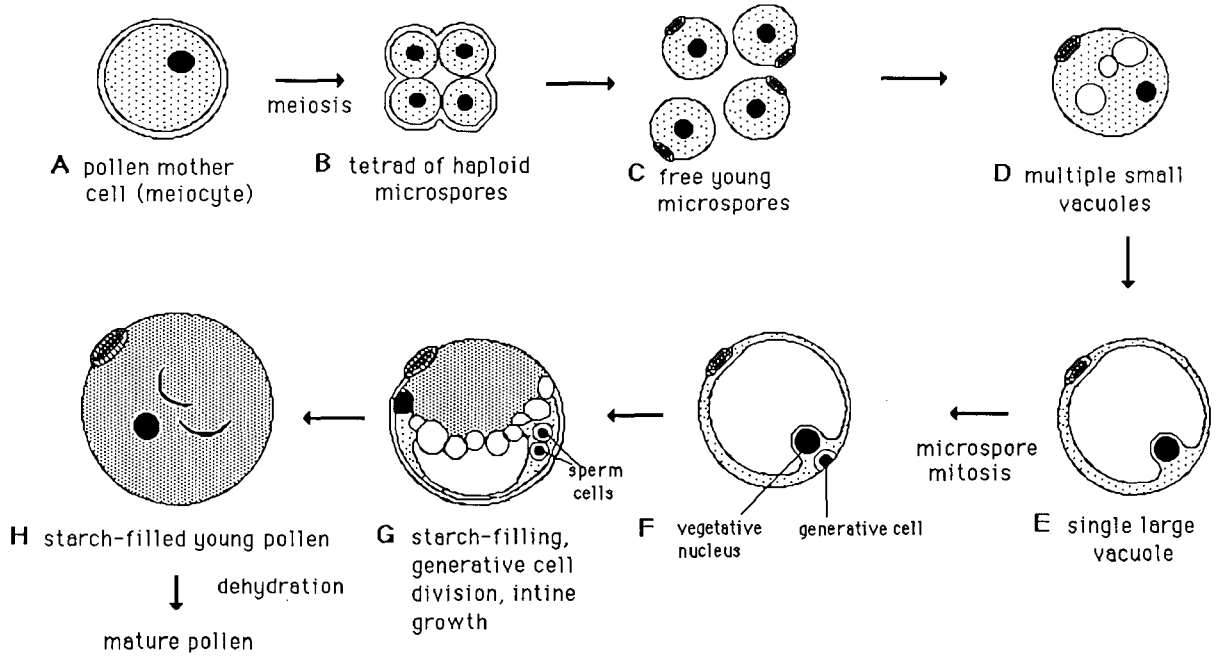

Figure 2. Schematic Diagram of Pollen Development in Maize.

Morphologically distinct stages of the developing pollen within the locule are depicted and are described in the text.

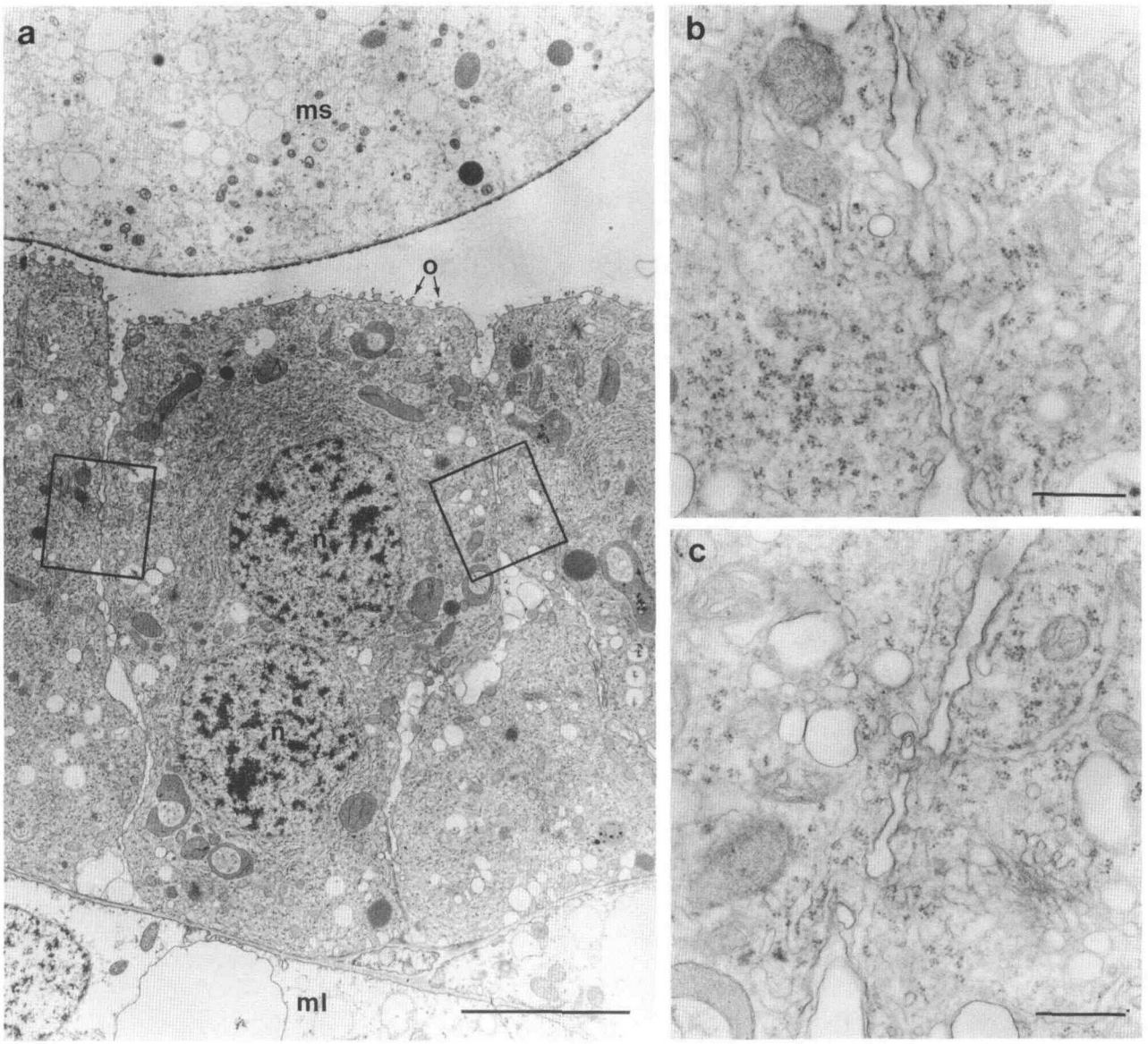

Figure 3. Tapetal Cell Structure.

(a) *Transmission electron micrograph of tapetal cells immediately after meiosis. ms, microspore; n, nucleus; o, orbicules, ml, middle layer. Bar = 5 μm.* **(b)** and **(c)**. Higher magnification views of boxed regions in **(a)** show the presence of cytoplasmic bridges. Bars = 0.5 μm.

system between the microspores and the tapetal cells? How is tapetal cell death controlled, and is it genetically programmed?

MEIOSIS

The male gametophyte of higher plants has provided a valuable cytological window through which to view the process of meiosis (Rhoades, 1950). For example, maize microsporocytes have been used in conjunction with well-characterized chromosomal translocations and inversions to address the mechanism of homolog pairing, or synapsis (Maguire, 1977). This work led to the proposal that chiasma formation (crossing over) is an integral part of the pairing mechanism rather than a consequence of synapsis. Recent molecular studies in other organisms support this model for homolog pairing. New optical technology is now being used in the three-dimensional reconstruction of the microsporocyte pachytene nucleus, including the identification of sites of chromosomal association with the nuclear envelope (K. Dawe and Z. Cande,

personal communication). If these studies can be extended to earlier meiotic stages, the relationship between crossing over and synapsis may be further clarified.

Genetic studies in maize have identified many mutants defective in meiosis (Golubovskaya, 1979). Mutants with particularly interesting phenotypes include those affecting chromosome behavior, such as *asynaptic* (reduced homolog pairing) and *sticky* (chromosomes stick together); those affecting spindle structure, such as *divergent spindle* (lack of focused spindle pole bodies in the first meiotic division); and those affecting control of cell division, such as *polymitotic* (supernumerary cytokinesis following the second division). The use of these mutants in conjunction with the cytological approaches described above should greatly enhance our understanding of fundamental meiotic processes, including how chromosome homologs pair, what factors might function specifically in meiotic as opposed to mitotic spindles, and how cytokinesis is normally coupled to chromosome replication and separation.

A UNIQUE CELL WALL

One of the most distinctive features of pollen grains is the pollen wall. The outermost wall, or exine, is ornamented in a species-specific fashion, as illustrated in Figure 4. The exine is composed largely of a material called sporopollenin. The extraordinary chemical resistance of sporopollenin was first reported more than 150 years ago (John, 1814), but its molecular structure is not yet understood. Until fairly recently, sporopollenin was thought to be a carotenoid polymer, but more recent inhibitor and physical data indicate that this is not the case (Prahl et al., 1985; Guilford et al., 1988). It is clear that sporopollenin is one of the most resistant biopolymers known; this property is presumably essential for the survival of the pollen grain free from the sporophyte prior to fertilization. The resistance of sporopollenin has been of great value to evolutionary and archeological researchers, who are able to use the distinctive pollen exine patterns to "sample" the plant community present at any particular time.

Exine biosynthesis is a joint effort on the part of tapetal cells and microspores. It is thought that the microspore (or even meiocyte) elaborates a surface template system known as the primexine or glycocalyx (Heslop-Harrison, 1971; Rowley, 1973) that determines the patterning of exine deposition. The mechanism by which primexine elements are distributed in a species-specific pattern in the microspore plasma membrane is currently unknown. The role of the tapetal cells in exine formation appears to be in the secretion of sporopollenin precursors that are then polymerized onto the template system. Orbicule structures composed of sporopollenin form on the locular face of the tapetal cells (Figure 3), probably as a byproduct of exine biosynthesis.

Although there have been a number of elegant morphological studies on pollen wall development (Skvarla and Larson, 1966; El-Ghazaly and Jensen, 1986), biochemical studies of this unique and highly specialized pollen structure are only beginning. Exine purification has allowed the production of specific antibodies (Southworth, 1988; Southworth et al., 1988) and the first characterization of potential structural proteins (Chay et al., 1992). The study of mutants defective in the formation of the exine (Morton et al., 1989) may provide additional approaches for understanding the synthesis, function, and basis of pattern formation of this unusual extracellular matrix.

MICROSPORE MITOSIS: A DEVELOPMENTAL SWITCH

After meiosis and an initial burst of growth and exine synthesis, the haploid microspores undergo a cytological reorganization in preparation for a key event in pollen development, the asymmetric division known as microspore mitosis. As is the case for many morphological events in plant development, an asymmetric division marks the initiation of a new developmental program. The pollen system provides a rare and exciting opportunity to study the cellular basis for the establishment and maintenance of polarity within a single cell. The cytological reorganization prior to microspore mitosis involves nuclear migration to a specific location near the cell periphery. In orchids (which lack the impermeable exine that interferes with immunolocalization studies), a novel microtubule system has been detected at the site of nuclear migration and subsequent division (Brown and Lemmon, 1991). In maize, the production of a large vacuole positions the entire cytoplasm in this region. If the role of organelles and cytoskeletal elements in establishing specific cytoplasmic domains in the microspore can be elucidated, the basis for many asymmetric morphogenic events in plant development may also be revealed.

An asymmetric, cone-shaped spindle perpendicular to the plasma membrane is utilized in this division (Brumfield, 1941; Brown and Lemmon, 1992). The nucleus adjacent to the plasma membrane becomes cellularized, forming the generative cell. Whereas the chromatin of the generative cell is highly condensed, the vegetative nucleus is larger, has more nuclear pores, and has decondensed chromatin, indicating that it is the transcriptionally active nucleus (LaFountain and LaFountain, 1973; Wagner et al., 1990). The basis for the striking differences in chromatin structure and activity in the two nuclei may lie in the components of the cytoplasmic domains established prior to division.

Microspore mitosis appears to be a critical point in commitment to the gametophytic pathway, analogous to other events in development where an aysmmetric division signals a commitment to differentiate, such as the first division in *Fucus* (Quatrano, 1990) and *C. elegans* (Strome, 1989) embryo development or the asymmetric division prior to guard cell differentiation (Cho and Wick, 1989). Anther and microspore culture experiments suggest that uninucleate microspores can be more easily induced than postmitotic young pollen to enter

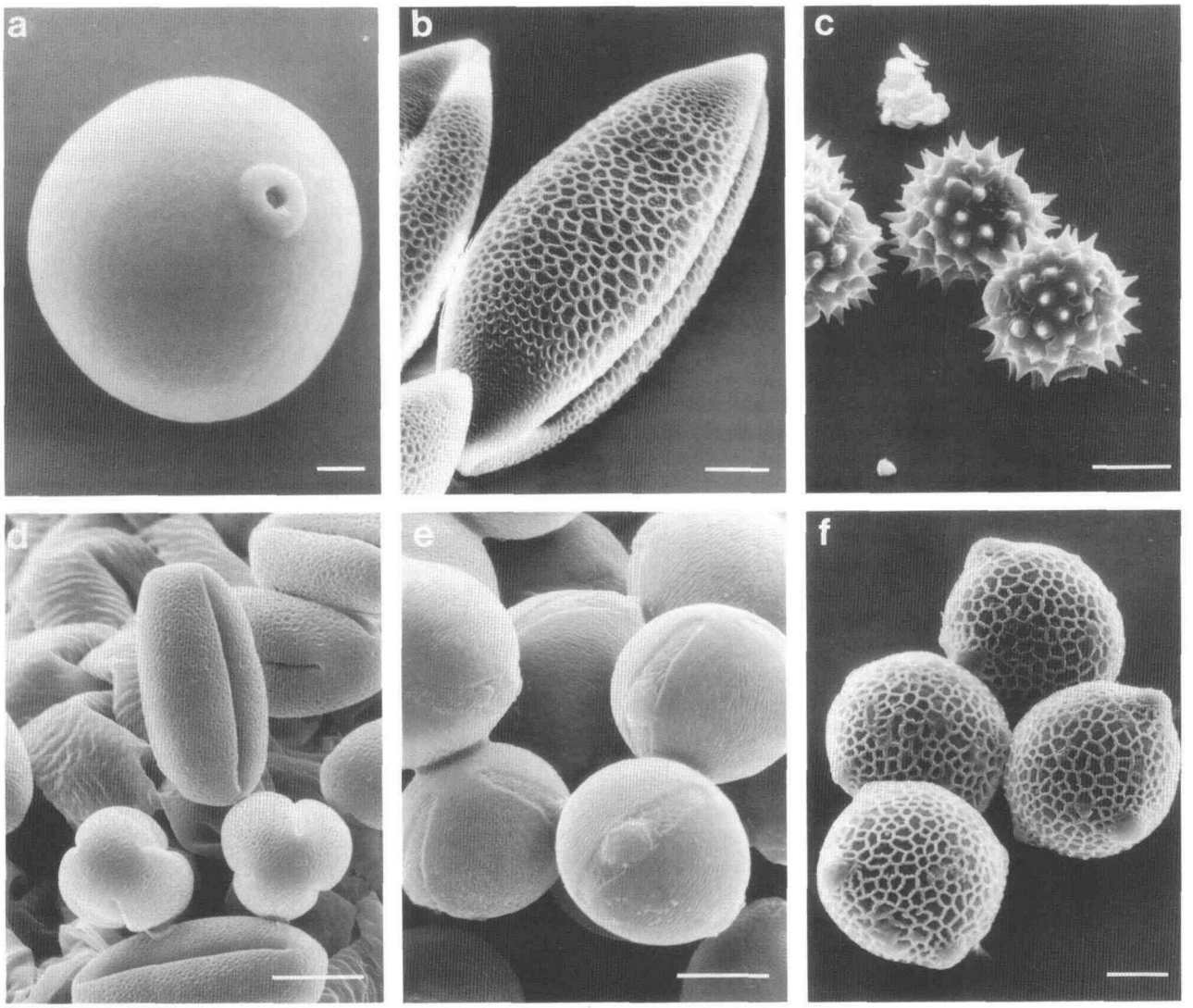

Figure 4. Scanning Electron Micrographs of Pollen.

(a) Maize.
(b) Amaryllis.
(c) Liatris, a composite.
(d) Redbud, a legume (with stigma).
(e) Tobacco.
(f) Impatiens.
Bars = 10 μm.

a sporophytic developmental program, producing androgenic structures (Gaillard et al., 1991). Studies of RNA and protein populations (Bedinger and Edgerton, 1990) and of protein synthesis in isolated microspores (Mandaron et al., 1990) indicate that a major developmental switch in gene expression occurs at this time. Studies of pollen-specific gene expression are consistent with this hypothesis in that the majority of such genes appear to become actively transcribed only after microspore mitosis (Stinson et al., 1987; McCormick, 1991). The role that the division of the microspore nucleus plays in the transcriptional activation of a gametophytic developmental program is an intriguing area for future research.

POLLEN TUBE GERMINATION AND GROWTH

Upon the interaction of the mature pollen with a stigma, the pollen hydrates and the pollen tube emerges from a pore on the exine surface. In the grasses, tube germination takes place

within a few minutes after the pollen lands on a silk. The pollen tube then elongates at astounding rates. For example, the rate of maize pollen tube growth can approach 1 cm/hr (Miller, 1919; Barnabas and Fridvalszky, 1984), a growth rate rivaled in biology only by neurite growth under certain conditions. Given this extremely rapid growth, it is likely that many of the transcripts and proteins that accumulate in the maturing maize pollen grain are synthesized in preparation for this effort. Very little is currently known about the "tip growth" mode of pollen tubes and root hairs in higher plants. Given that tip growth consists of the rapid polar extension of a single cell, there could be fundamental differences between this mode of growth and the standard mode of cellular growth in plants. However, the recent discovery of a hydroxyproline-rich glycoprotein-like gene expressed in maturing maize pollen suggests that pollen tube growth, like that of other plant cells, could involve hydroxyproline-rich glycoprotein deposition during wall formation (A. Broadwater, K. Lowrey, A. Rubinstein, and P. Bedinger, unpublished data).

As the pollen tube elongates, the pollen cytoplasm, vegetative nucleus, and sperm cells are transported within the tip of the pollen tube through the transmitting tissue to the ovule. This process, therefore, represents an extremely rare and striking example of cell migration in plant development (Sanders and Lord, 1989). In the case of maize, these structures may migrate over 30 cm within about 24 hr—a truly remarkable feat.

FUTURE DIRECTIONS

As we look to the future use of genetic engineering in agriculture, the control of plant reproduction has a high priority. Therefore, it is essential that we understand the biological mechanisms that determine fertility. In addition to the practical applications of pollen research, the pollen developmental pathway is an appealing system for more basic investigation. Pollen development is a relatively simple system that comprises many fundamental processes. For example, the interactions between the tapetal cells and the developing gametophytes may model the interactions that take place between cells in more complex systems. Previous views that the tapetal cells act simply to provide nutrition to the microspores need to be modified to include more subtle and varied roles in microsporogenesis, such as the cooperative construction of an extracellular structure, the exine. The study of tapetal cell differentiation, sporophytic and gametophytic male sterility genes, and exine biosynthesis should shed light on the mechanisms involved in these interactions with the microspores.

Another key component in development and differentiation is the establishment and maintenance of the cellular domains that lead to polarization of cells. Polarity plays an essential role in pollen development at several points, including microspore mitosis and pollen tube growth. Cytological and molecular studies of these processes could elucidate basic mechanisms in polar cellular organization. In addition, the availability of mutants and molecular tools makes microsporogenesis an excellent system for the study of the mechanism of chromosome homolog pairing during meiosis and the molecular basis of developmental switches.

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